HYDROGEN EXCHANGE IN THE HYDROPHILIC REGIONS OF DETERGENT-SOLUBILIZED M13 COAT PROTEIN DETECTED BY ^{13}C NUCLEAR MAGNETIC RESONANCE ISOTOPE SHIFTS

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^{13}C nuclear magnetic resonance (NMR) spectroscopy has been used as an indirect probe of the exchange rates of selected amide protons in the detergent-solubilized coat protein of the filamentous coliphage M13. Although the phage particle contains no lipid, the 50-residue major coat protein is inserted as an integral protein in the inner membrane of the host cell during infection (1). It is as a model membrane protein; the sequence (below) shows a highly hydrophobic central core flanked by acidic NH_2-terminal and basic COOH-terminal regions (2, 3):

hydrophilic
AEGDDPAKAAFDSLQASATE

hydrophobic
YIGYAWAMVVVIVGATIGI

hydrophilic
KLFKKFTSKAS

The lifetimes of protein amide protons may vary from seconds to many months. Most measurements involve direct determination of the rate of equilibration with deuterium- or tritium-substituted water. However, rapidly exchanging protons are usually lost before data can be obtained. We have applied an indirect equilibrium approach to the measurement of exchange rates by exploiting the small two-bond isotope shift (~0.09 ppm) experienced by the carbonyl carbon of a peptide bond after deuteration of the adjacent amide nitrogen atom (4, 5). The amide protons of M13 coat protein, as observed by ^1H NMR, are poorly resolved and thus difficult to quantitate (6). Our method allows us to examine the behavior of individually assigned amide protons that are exchanging over a wide range of rates, including those which are too fast for detection by nonequilibrium procedures.

RESULTS AND DISCUSSION

M13 coat protein was selectively labeled at the carbonyl carbons of lysine, proline, or phenylalanine by inclusion of the appropriate [^{13}C] enriched amino acid in the culture medium. These carbonyl labels provide information about the amide of the succeeding residue; e.g., Lys-48 reflects exchange at Ala-49. There are five lysines in the molecule (residues 8, 40, 43, 44, and 48), three phenylalanines (residues 11, 42, and 45), and a single proline (residue 6); thus the present labels are concentrated in the hydrophilic ends. The amphiphilic nature of the protein necessitates the use of solubilizing detergents. Sodium dodecyl sulphate (SDS) was chosen for these experiments because it forms discrete coat-protein–containing micelles (7), has a low pK_a so that the pH may be varied, and results in carbonyl linewidths that are narrow. Other detergents, such as sodium deoxycholate (DOC), which are considered less "denaturing," result in very broad lines for some resonances, strongly suggestive of conformational heterogeneity. The coat protein is dimeric in both detergents (7).

Fig. 1 illustrates the two-bond deuterium isotope effect for coat protein in SDS micelles at pH 9.0 containing lysine labeled with ^{13}C at C1. Integration of the peaks labeled *A* to *E* in a fully relaxed spectrum obtained in the absence of the nuclear Overhauser enhancement is consistent with five residues, two of which (peaks *C* and *D*) occur at very similar chemcial shifts. Carboxypeptidase A digestion has

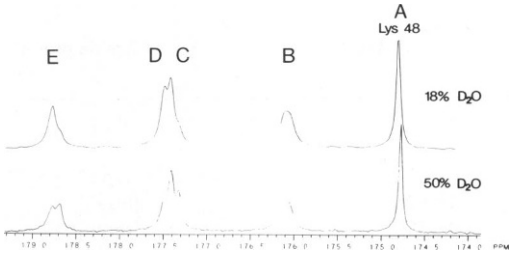

FIGURE 1 Carbonyl region of the 75 MHz proton decoupled ^{13}C NMR spectrum of 1-[^{13}C]-lysine labeled coat protein in 50% D_2O and 18% D_2O. Samples were 0.8 mM protein in 10 mM SDS 33 mM sodium borate pH 9.0 in a 12 mm tube. Spectra (30,000 scans) were collected at 23°C using a sweep width of 1,000 Hz and 4K data points. Chemcial shifts are given relative to tetramethylsilane at 0 ppm.

shown peak *A* to correspond to lysine 48, and peak *B* was assigned to lysine 8 in the analogous spectrum obtained in DOC micelles.[1] The remaining lysines are unassigned but close together in the sequence.

Carbonyl carbon resonances in a 1:1 H_2O:D_2O mixture are split into a CONH peak (downfield) and a COND peak (upfield) if the exchange rate is slow enough. The splitting is 6–7 Hz (~0.09 ppm at 75 MHz); slow exchange corresponds to an exchange rate of $<40\ s^{-1}$. At faster rates ($>40\ s^{-1}$) the splitting collapses into a single peak at an average chemical shift according to the isotopic composition of the solvent. The lysine carbonyls record a range of exchange conditions: peaks *C*, *D*, and *E* are in the slow exchange limit and peak *A* (lys 48) is in fast exchange with the solvent. Peak *B* is unusually broad and difficult to analyze.

Peptide proton exchange is catalyzed by H^+ or OH^- according to the following equation:

$$k_{exch} = k_{OH}[OH^-] + k_H[H^+], \quad (1)$$

where $k_{OH} = 10^7\ M^{-1}s^{-1}$ and $k_H = 10^{-1}\ M^{-1}s^{-1}$ (8) for a freely exposed peptide at 0°C. Thus the minimum is observed at pH ~3. Above this pH, the rate is proportional to $[OH^-]$, increasing 10-fold for each unit rise in pH. Resonances may thus be brought from slow to fast exchange simply by increasing the pH (5); this is illustrated for 1-[^{13}C]-phenylalanine-labeled protein in Fig. 2. A similar type of pH profile is observed for 1-[^{13}C]-proline–labeled protein (spectrum not shown). Resonance *A* in Fig. 2 has been assigned to Phe 11 in DOC micelles,[1] but has not been assigned directly in SDS. The pH-dependent chemical shift changes of peaks *A* and *B* might be related to the protonation states of Asp 12 and Lys 43.

[1]Henry, G. D., J. H. Weiner, and B. D. Sykes. 1985. ^{13}C NMR spectroscopy of M13 coat protein in detergent micelles: assignment of labeled carbonyl resonances. Manuscript in preparation.

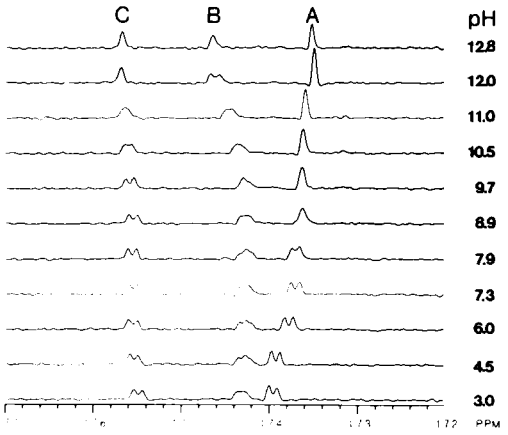

FIGURE 2 pH titration of 1-[^{13}C]-phenylalanine-labeled coat protein in 50% D_2O, 8,000 scans per spectrum. Conditions are otherwise as given in Fig. 1.

Peak *B* actually exists as two overlapping peaks in H_2O (data not shown), which may be related to the dimeric nature of the protein. In 50% D_2O it appears as two overlapping doublets. The pH values at which the collapse of the splitting occurs correspond to a rate of $40\ s^{-1}$, which can be correlated with a freely exposed peptide N–H according to Eq. 1. The difference in temperature was corrected using an activation energy of 17.5 kcal mol^{-1}. The results are summarized in Table I, which shows all amide hydrogens to be exchanging at slower rates than those observed for a small peptide (5).

The rate of exchange of amide protons in proteins depends on solvent accessibility, which in turn results from secondary structure and dynamic fluctuations (8). The hydrophilic regions of detergent-bound M13 coat protein display a range of exchange rates varying by as much as 5×10^4. The NH_2-terminal region (up to Asp 12) appears relatively well-exposed to the solvent, as does Ala-49 at the other end. The COOH-terminal Lys-Phe cluster, (residues 41–46), by contrast, is much less accessible, and may well

TABLE I
HYDROGEN EXCHANGE PARAMETERS

Carbonyl label	Amide proton	pH of initial collapse	Exposure (relative to free peptide)
pro 6	ala 7	8.2	0.1
phe 11	asp 12	8.4	0.1
{phe 42, phe 45}	{lys43, thr46}	{10.1, <9.3}	{0.002, 0.01}
lys 8	ala 9	8.7	0.05
lys 48	ala 49	7.3	1
{lys 40, lys 43, lys 44}	{leu 41, lys 44, phe 45}	>10.0, >10.0, >11.9	{0.002, 0.002, 0.00002}

participate in some hydrogen bonded structure. It is possible, of course, that the high pH values used in obtaining these data may have led to partial denaturation of the molecule (so that even slower exchange rates exist in reality), although the chemical shifts of the carbonyl carbons were not severely affected by high pH values. Circular dichroism data (9) have suggested that 80% of SDS-bound protein exists as α or β structure.

The use of SDS (which typically denatures proteins) as a solubilizing agent can be defended on the grounds of the similar CD spectra obtained with M13 coat protein in SDS, DOC, and phospholipids (9); the latter are presumed to represent a native environment. ^{13}C chemical shifts and T_1 relaxation times determined for a number of labeled carbon atoms in SDS and DOC (a milder detergent) are similar. Furthermore, the coat protein is an atypical protein, being both small and highly hydrophobic.

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REFERENCES

1. Smilowitz, H. 1974. Bacteriophage f1 infection: fate of the parental major coat protein. *J. Virol.* 13:94–99.
2. Asbeck, V. F., K. Beyreuther, H. Kohler, G. von Wettstein, and G. Braunitzer. 1969. Die Konstitution des Hullproteins des Phagen fd. *Hoppe-Seyler's Z. Physiol. Chem.* 350:1047–1056.
3. Nakashima, Y., and W. Konigsberg. 1974. Reinvestigation of a region of the fd bacteriophage coat protein sequence. *J. Mol Biol.* 88:598–600.
4. Feeney, J., P. Partington, and G. C. K. Roberts. 1974. The assignment of carbon-13 resonances from carbonyl groups in peptides. *J. Magn. Reson.* 13:268–274.
5. Hawkes, G. E., E. W. Randall, W. E. Hull, D. Gattegno, and F. Conti. 1978. Qualitative aspects of hydrogen deuterium exchange in the ^{1}H, ^{13}C, and ^{15}N nuclear magnetic resonance spectra of viomycin in aqueous solution. *Biochemistry.* 19:3986–3992.
6. Cross, T. A., and S. J. Opella. 1980. Structural properties of fd coat protein in sodium dodecyl sulphate micelles. *Biochem. Biophys. Res. Commun.* 92:478–484.
7. Makino, S., J. L. Woolford, C. Tanford, and R. E. Webster. 1975. The interaction of deoxycholate and detergents with the coat protein of bacteriophage f1. *J. Biol. Chem.* 250:4327–4332.
8. Englander, S. W., and N. R. Kallenbach. 1984. Hydrogen exchange and structural dynamics of proteins and nucleic acids. *Quart. Rev. Biophys.* 16:521–655.
9. Nozaki, Y., B. K. Chamberlain, R. E. Webster, and C. Tanford. 1976. Evidence for a major conformational change of coat protein in assembly of f1 bacteriophage. *Nature (Lond.).* 259:335–357.